26

Plasmodium knowlesi Sinton and Mulligan, 1932

IT is not unlikely that Franchini (1927) was the first person to see *Plasmodium knowlesi* and to recognize that the parasite he saw in the blood of *Silenus cynomolgus* (= *Macaca fascicularis)* was different from *P. inui* and *P. cynomolgi.* Later (1931) it was seen by Dr. H. G. M. Campbell who was working in kala-azar and had no particular interest, at the time, in the plasmodium he encountered in *M. fascicularis.* Dr. Napier, on the other hand, with whom Dr. Campbell was working, drew blood and inoculated it into 3 other monkeys, one of which was a rhesus; it developed a fulminating infection. The original monkey was given to Dr. Das Gupta who maintained the strain for some time by subpassage (see Knowles, 1935). Napier and Campbell (1932) investigated the tendency for the parasite to produce hemoglobinuria in *Cercopithecus pygerythrus* (actually, *M. fascicularis)* and *M. rhesus* (= *M. mulatta).* In the same year (1932) Knowles and Das Gupta described the blood forms of the parasite and showed that it could be transmitted to man. From this vantage point, one wonders why neither group elected to name the parasite. It must be remembered, however, that not all investigators are taxonomic addicts and, too, maybe they recognized that the literature on these parasites was already in a state of disorganized chaos and elected to leave the naming to "the brave." Sinton and Mulligan (1932), after studying the Knowles and Das Gupta material and their own isolate from a *M. fascicularis,* obtained in Singapore, noted the distinctive stippling in the red cells, the presence of an accessory dot, and the 24-hour schizogonic cycle which convinced them that the parasite represented a new species. They gave it the name *Plasmodium knowlesi* in honor of Dr. R. Knowles. In 1935, Mulligan wrote a more detailed description of the parasite accompanied by a well executed plate which gave increased stature to the parasite's distinctive nature.

Malariologists have puzzled over a paper by Ionesco-Mihaiesti *et al* (1934) in which they claimed to have found *P. inui* in the blood of a baboon. The parasite was said not only to infect rhesus but, also, that it would infect man. Baboons are not infected naturally with malaria and until recently, *P. inui* failed to grow in man. The puzzle was cleared up in 1964 when Professor Garnham visited Roumania and, through the kindness of Dr. G. Lupascu, who had kept the original slides, was able to examine the original material; the parasite in question was actually *P. knowlesi.* The monkey-to-man passage was thus cleared up because *P. knowlesi* will infect man as first shown by Knowles and Das Gupta (loc. cit.). The baboon had been given inoculations of emulsified spleen and other organs from a *M. fascicularis,* the natural host of *P. knowlesi,* which would account for its infection. The infection in the baboon was recognized as mild which might be expected of an abnormal host except, as was shown in this laboratory, *P. knowlesi* will kill baboons when infection is induced through the inoculation of parasitized blood.

The true home of *P. knowlesi* is peninsular Malaysia where monkeys, especially *M. fascicularis,* are commonly infected. Their infections may include species other than *P. knowlesi* and their separation may require the employment of several techniques and more than a dash of patience. Its range extends east to the Philippines (Lambrecht *et al,* 1961) and

north to Taiwan (Yokagawa *et al,* 1941). If careful surveys were made, it probably would be found in Java, southern Thailand, and possibly in similar climatic areas in Cambodia and South Vietnam.

From time to time, variants and/or strains, or subspecies, of *P. knowlesi* have been isolated and described. Sinton and Mulligan (1933) isolated 5 different strains, but found no significant points of difference between them and their original strain. In 1953, Edeson and Davey isolated a strain from a *M. fascicularis* trapped in Negri Sembilan, Malaya; which, following studies there, in India, and in England, turned up no features that would distinguish it. The strain isolated directly from *Anopheles hackeri* (Wharton and Eyles, 1961) is now known as the 'hackeri' strain and it, too, behaves like the earlier isolates.

Among the variants, the first to be described was by Brug (1934) who described variety *sintoni* from a *M. fascicularis* (actual source is unknown but credited by some authors to Java) which he considered different from the typical *P. knowlesi.* The distinguishing characteristics were absence of cellular distortion, rod-shaped pigment, and red-staining rims around the schizonts which sometimes extended as septa between the merozoites. No other like material has come to hand and so, for the present, judgment is withheld as to whether *sintoni* is a valid form.

Yokagawa (1941) gave the variety name *arimai* to the parasite seen by Arima (1933) in blood from a *M. cyclopis,* the only species of monkey found on Taiwan. In the same paper, Yokagawa offered the name *Plasmodium taiwanensis* for a new species which he said had an asexual cycle of 11 to 24 days. As one reviews the literature, difficult at best, but cleared up somewhat by Hsieh (1960), it would appear that var. *arimai* was described again by Yokagawa *et al* (1941, 1942) and Yokagawa (1942, 1942a). In 1951 according to Hsieh (loc. cit.), Yokagawa mentioned that *P. knowlesi* var. *arimai* was close to *P. knowlesi* but that it would not infect man. Because the data on the length of its asexual cycle is in doubt, its low pathogenicity to monkeys, and its failure to grow in man, the parasite is most likely *P. inui.* The species *taiwanensis* is surely a *Hepatocystis* to which, according to Hsieh (loc. cit.), Garnham agrees. Another species variety *Plasmodium cynomolgi cyclopis* Inoki *et al,* 1942 with *knowlesi* affinities has also been described from Taiwan; it is discussed in Chapter 6. Because so little is known about the malarias on Taiwan, including a complete blank on the vectors, it is hoped that investigators will find time to pursue the problem there.

This leaves us with *P. knowlesi edesoni* Garnham, 1963. The parasite exhibits a quotidian cycle with near absence of schizogony in the peripheral blood, reminiscent of *P. coatneyi* and *P. falciparum* up to the appearance of gametocytes which are spherical as against crescentic in *P. falciparum.* It is infectious to rhesus monkeys, many of which recover unless splenectomized.

The rings appear in the circulation about midnight and many of them carry an accessory chromatin dot; multiple infections may appear. As growth proceeds, the parasites become drawn out, stretching across the host cell with the nucleus on one side of the band. In late evening, the more compact parasites begin to leave the peripheral circulation only to disappear completely about 3 hours before sporulation pours young forms into the circulation again. The mature schizonts, with condensed pigment, carry in the neighborhood of 12 merozoites.

The mature macrogametocytes occupy the entire red cell with a nucleus larger than ordinary; dark pigment is scattered in the cytoplasm. The adult microgametocytes take up most of the host cell and support a large red-staining nucleus which may be surrounded by a thick rim of pigment.

It is unfortunate that this strain is no longer available to allow for comparison of the sporogonic and other cycles with classical *P. knowlesi* and with *P. coatneyi.* Because the original infection in *M. fascicularis* came from an area near Kuantan, Pahang, Malaysia, it is hoped that it can be re-isolated. Until overall comparisons can be made, the subspecies is considered valid.

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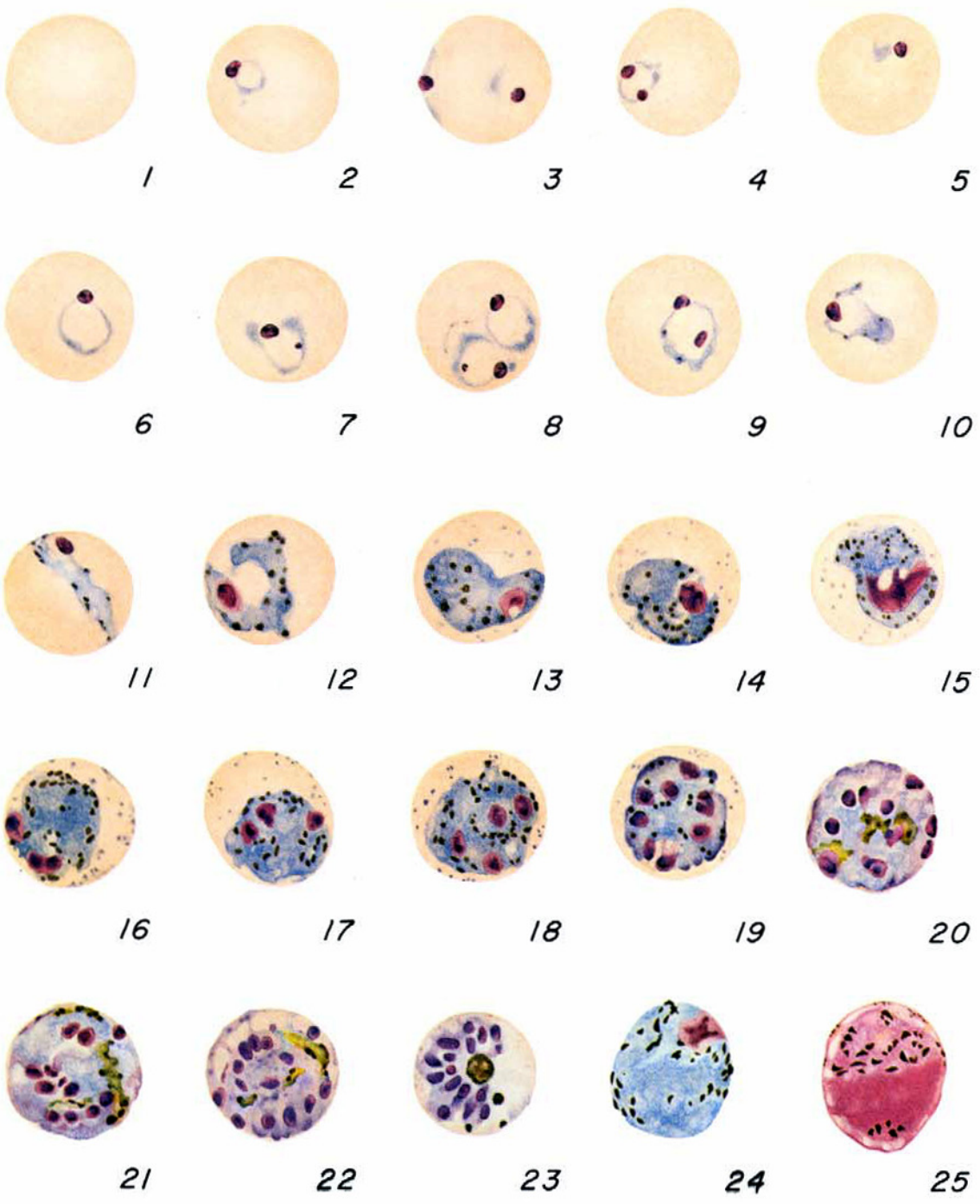

PLASMODIUM KNOWLESI

Cycle in the Blood

PLATE LI

The young ring forms in the rhesus monkey and in man may appear in large numbers in the circulating blood. They resemble *P. falciparum* rings but their nucleus is spherical and prominent, many times lying inside the ring. Appliqué forms appear (Fig. 3) along with regular rings harboring one or more accessory chromatin dots (Figs. 4, 7-9). Sinton and Mulligan (1933) regarded the latter as diagnostic of *P. knowlesi* but we know now that these structures occur in other simian forms, too. When full grown, the non-amoeboid rings may occupy half or more of the host erythrocyte. At this stage of growth, band forms appear, reminiscent of *P. malariae* (Fig. 11). With the loss of its vacuole, the parasite shrinks, becomes compact, and pigment appears in the form of dark grains; the nucleus increases in size, and takes a deep red stain. The cytoplasm stains a deep blue. The host erythrocyte shows stippling which some authors have called 'Sinton and Mulligan's' stippling, since it is not of the Schüffner type (Figs. 13-18). With the advent of schizogony, the nucleus divides and the process continues until as many as 16 merozoites, average 10, are produced. The process of schizogony results in some contraction of the parasite (Fig. 19) but with further development, it eventually fills the host cell (Fig. 20). At first, the pigment is scattered but now collects into one or more yellowish-black masses, and eventually into a single mass in the mature schizont (Fig. 23).

The early sexual forms may be recognized as small solid bodies which appear to grow more slowly than the asexual forms consuming probably 48 hours to complete their development. This parasite like some other species, notably *P. eylesi* and *P. jefferyi,* displays a striking color difference in the sexual forms (Figs. 24, 25). The mature macrogametocyte is generally spherical and fills the host cell which may be enlarged to a diameter of 8.5 µ. The cytoplasm stains a distinctive blue and the nucleus, placed eccentrically, takes a deep pink stain enclosing a heavier stained irregular area. The black pigment granules are prominent and scattered irregularly in the cytoplasm (Fig. 24). The microgametocyte is sometimes smaller than the distaff parasite but this is not always true. The cytoplasm stains a medium pink with the nucleus a darker shade. The nucleus makes up about one-half the body of the parasite and which is without pigment granules. The latter are jet black and scattered in the cytoplasm (Fig. 25).

The asexual cycle in the blood occupies 24 hours, the only example of a quotidian cycle among the primate malarias.

Sporogonic Cycle

PLATE LII

The development of *Plasmodium knowlesi* to the point of sporozoite-positive salivary glands has been reported in *Anopheles annularis* (Sinton and Mulligan, 1933; Singh *et al,* 1949), in *A. aztecus* (Garnham *et al,* 1957), in *A. stephensi* (Singh *et al,* 1949; Hawking and Mellanby, 1953; Hawking *et al,* 1957; and Garnham *et al,* 1957), in *A. atroparvus* (Weyer, 1937; Hawking *et al,* 1957), and in *A. b. balabacensis* and *A. freeborni* (Collins *et al,* 1967). In *A. stephensi* and *A. atroparvus,* the oocysts developed on the guts but sporozoites were rarely found in the salivary glands. In our studies, we have followed the sporogonic development in *A. b. balabacensis, A. freeborni, A. maculatus, A. quadrimaculatus,* and *A. atroparvus* (Table 40).

In *A. b. balabacensis,* at day 4, the mean

PLATE LI.—*Plasmodium knowlesi*.

Fig. 1. Normal red cell.
Figs. 2-9. Young trophozoites.
Figs. 10-12. Growing trophozoites.
Figs. 13-15. Mature trophozoites.
Figs. 16-23. Developing schizonts, nearly mature, and mature schizonts.
Fig. 24. Mature macrogametocyte.
Fig. 25. Mature microgametocyte.

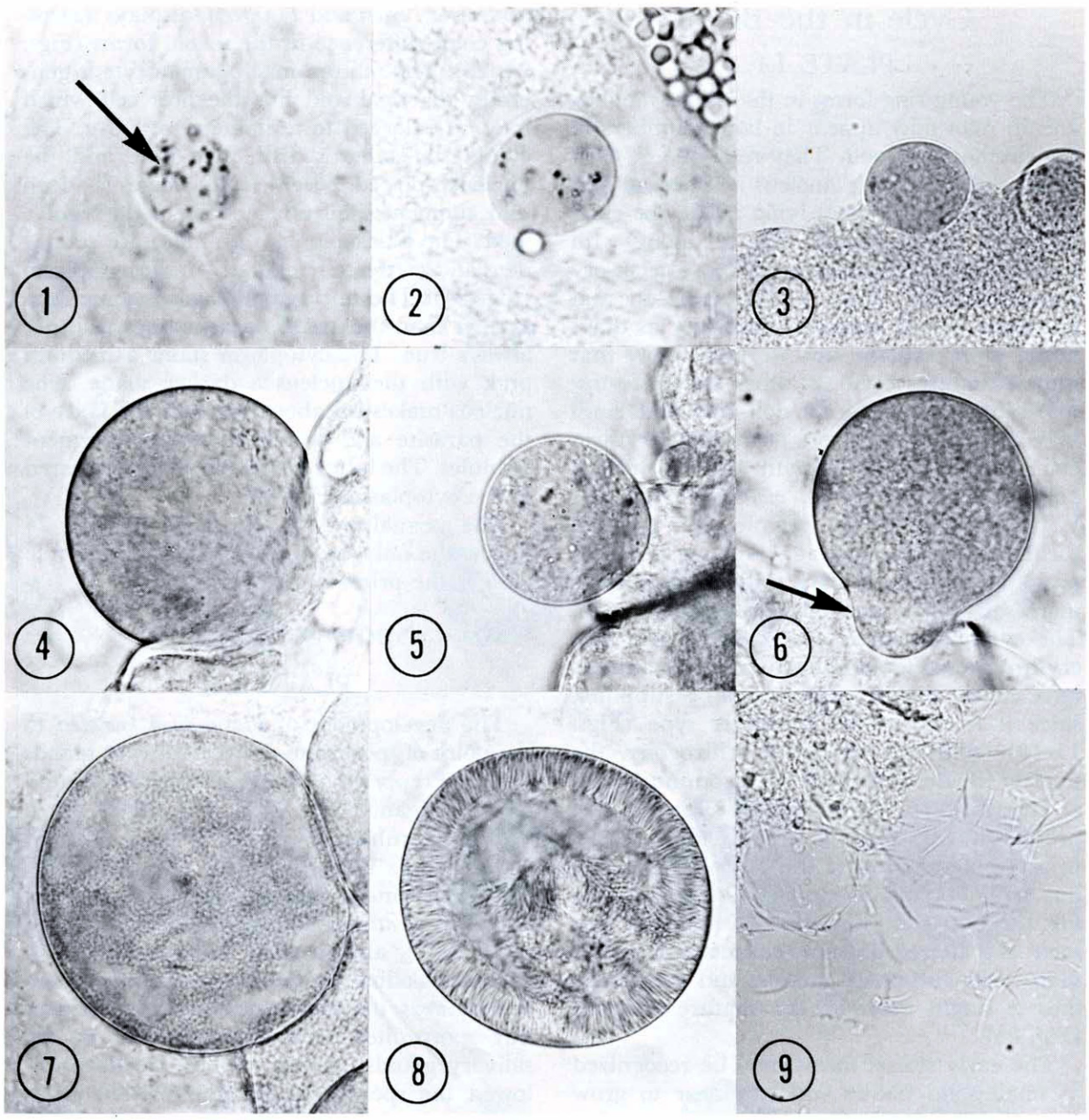

PLATE LII.—Developing oocysts and sporozoites of *Plasmodium knowlesi* in *Anopheles b. balabacensis* mosquitoes. X 580.

Fig. 1. 4-day oocyst showing scattered pigment. X 1300.
Fig. 2. 5-day oocyst. X 1300.
Fig. 3. 7-day oocyst.
Fig. 4. 8-day oocyst.
Fig. 5. 9-day oocyst.
Fig. 6. 10-day oocyst showing peduncle.
Fig. 7. 11-day oocyst showing early differentiation.
Fig. 8. 11-day fully differentiated oocyst.
Fig. 9. Sporozoites near salivary gland tissue.

oocyst diameter was 8 μ, with a range of 5 to 12 μ. The oocysts continued to grow and by day 10, the mean size was 62 μ, with a range of 18 to 103 μ sporozoites were present at this time in the salivary glands.

In *A. freeborni* and *A. maculatus,* the oocysts developed, but the mean diameters were smaller than in *A. b. balabacensis.* In addition, the sporozoites, although present in the salivary glands of both species at day 12, were very scarce. The oocysts in *A. quadrimaculatus* were actually larger on comparable days than were those in the *A. b. balabacensis.* Sporozoites were present in the salivary glands on day 11. Sporozoites were not found in *A. atroparvus* although dissections were carried out through day 11. The extrinsic incubation periods in the mosquitoes ranged from 12 to 15 days (mean of 13.0 days). The sporozoites were shown to be infective in that transmission was obtained, by bites of *A. b. balabacensis* mosquitoes, in rhesus monkeys on 30 occasions. The prepatent periods ranged from 6 to 9 days (mean 7.1 days). On 9 other occasions, dissected guts and glands of *A. b. balabacensis* (2 times), *A. freeborni* (6 times), and *A. maculatus* (once) were inoculated into rhesus monkeys. The prepatent periods under these conditions ranged from 5 to 12 days with a mean of 7.2 days.

A comparison of the growth curves of *P. knowlesi* and *P. cynomolgi* in *A. b. balabacensis* mosquitoes (Fig. 70) indicates a close similarity between the two. It is surprising that the tertian parasite *(P. cynomolgi)* and the quotidian parasite *(P. knowlesi)* should have similar growth patterns when the growth phases in the blood and in the fixed tissue are so dissimilar.

Cycle in the Tissue

PLATE LIII

The tissue forms of *P. knowlesi,* like the other primate forms, develop in the parenchyma cells of the liver and display structures which appear to be highly distinctive. Certain stages in the exoerythrocytic cycle were demonstrated by Garnham *et al,* 1957.

The earliest forms were seen at 92 hours after infection. At that stage, they occupied most of the enlarged host cell with the parasite oval in shape and with a smooth outline. The most arresting feature of the interior was the decided separation of chromatin and cytoplasm with the latter condensed into flocculi. Vacuoles were present. The nuclei were large, numerous, and appeared as an aggregate of chromatin dots. In the main, there was no continuity between nucleus and cytoplasm. The smallest EE bodies measured 11 x 21 μ and the largest 29 x 29 μ. Only one 117-hour (4¾ days) form was seen. It was an oval parasite which measured 33 x 50 μ. In appearance, this form was much like the

TABLE 40.—Oocyst diameters of *Plasmodium knowlesi* in *Anopheles b. balabacensis, A. freeborni, A. maculatus, A. quadrimaculatus*, and *A. atroparvus.*

Days after Infection	*A. b. balabacensis*			*A. freeborni*			*A. maculatus*			*A. quadrimaculatus*			*A. atroparvus*		
	No.	Range*	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
4	72	5-12	8												
5	246	8-24	15	145	8-22	14	36	8-18	14				52	12-18	14
6	266	11-35	20	306	11-38	19	56	12-28	19	6	26-41	31	166	12-32	19
7	244	12-53	34	155	9-60	33	177	9-50	23	28	24-47	40	72	20-53	33
8	226	14-77	45	215	14-63	39	129	18-63	37	19	35-74	55	113	19-64	42
9	309	13-92	57†	190	13-87	51†	155	18-74	43†	134	22-78	51†	75	24-74	42
10	195	18-103	62†**	242	18-101	58†	279	14-81	47†	122	25-100	72†	144	20-87	54
11	199	24-100	67†**	83	26-106	64†	136	27-89	53†	88	27-99	71†**	10	52-89	78†
12	50	27-79	58†**	5	44-67	54†**	104	20-83	53†**						
Totals	1907	5-103		1341	8-106		1072	8-89		397	22-100		632	12-89	

* Measurements expressed in microns.
† Oocyst differentiation.
** Sporozoites present in the salivary glands.

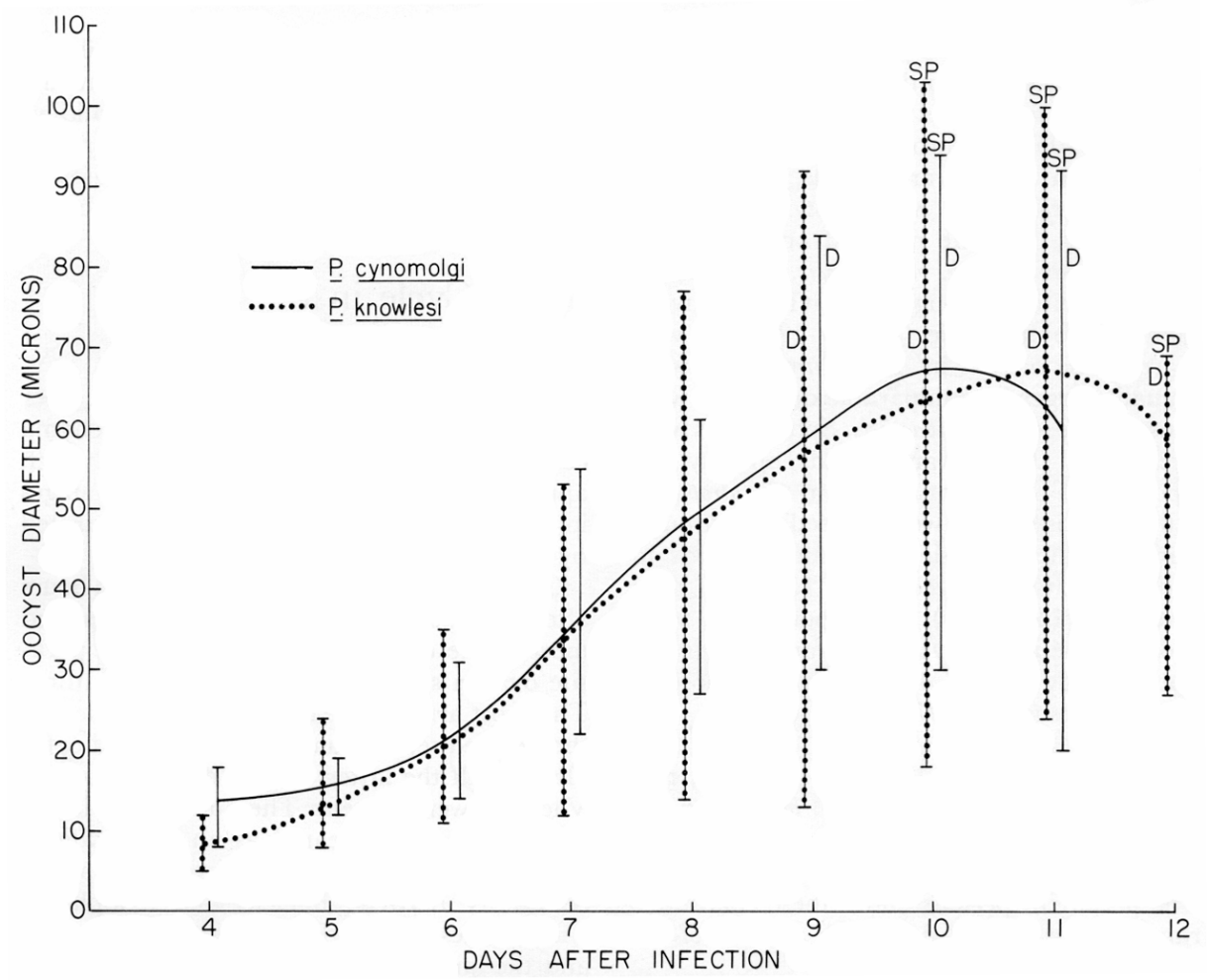

FIGURE 70.—Range in oocyst diameters and the mean oocyst diameter curve of *Plasmodium knowlesi* and *P. cynomolgi* in *Anopheles b. balabacensis* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

earlier ones except that vacuoles were absent.

The biopsy material at 124 hours (5¼ days) caught the parasites just prior to the final division to form merozoites because 14 hours later, ring forms were in the circulating blood; they appeared to be about 8 hours old.

The EE bodies were easily recognized because of their large size. They were oval bodies with an even border and prominent clefts or spaces in the cytoplasm. Sometimes these parasites were pear- or hourglass-shaped. Cytoplasmic flocculi were present, and the striking feature was the early differentiation of the cytoplasm which is not seeded with nuclear material until later. The nuclei of these 5-day forms appeared to be of 3 types: clusters of dots, very small dots of chromatin scattered in the cytoplasmic masses, and bars. The size of the EE bodies ranged from 38.2 x 25.5 μ to 52 x 52 μ.

These authors also found a 141-hour (5¾ days) ruptured form surrounded by phagocytes. The infection had become patent some hours before. The EE body area was approximately 75 x 110 μ; only a few merozoites were seen in the center of the area.

In our own studies, we have infected monkeys with the *A. hackeri* strain of *P. knowlesi* following the technique of Held *et al* (1966) in which infected salivary glands from *A. b. balabacensis* mosquitoes are injected directly into the liver. Beginning at 48 hours after injection, biopsies were taken at 8 hour intervals through 120 hours. Studies of the sections

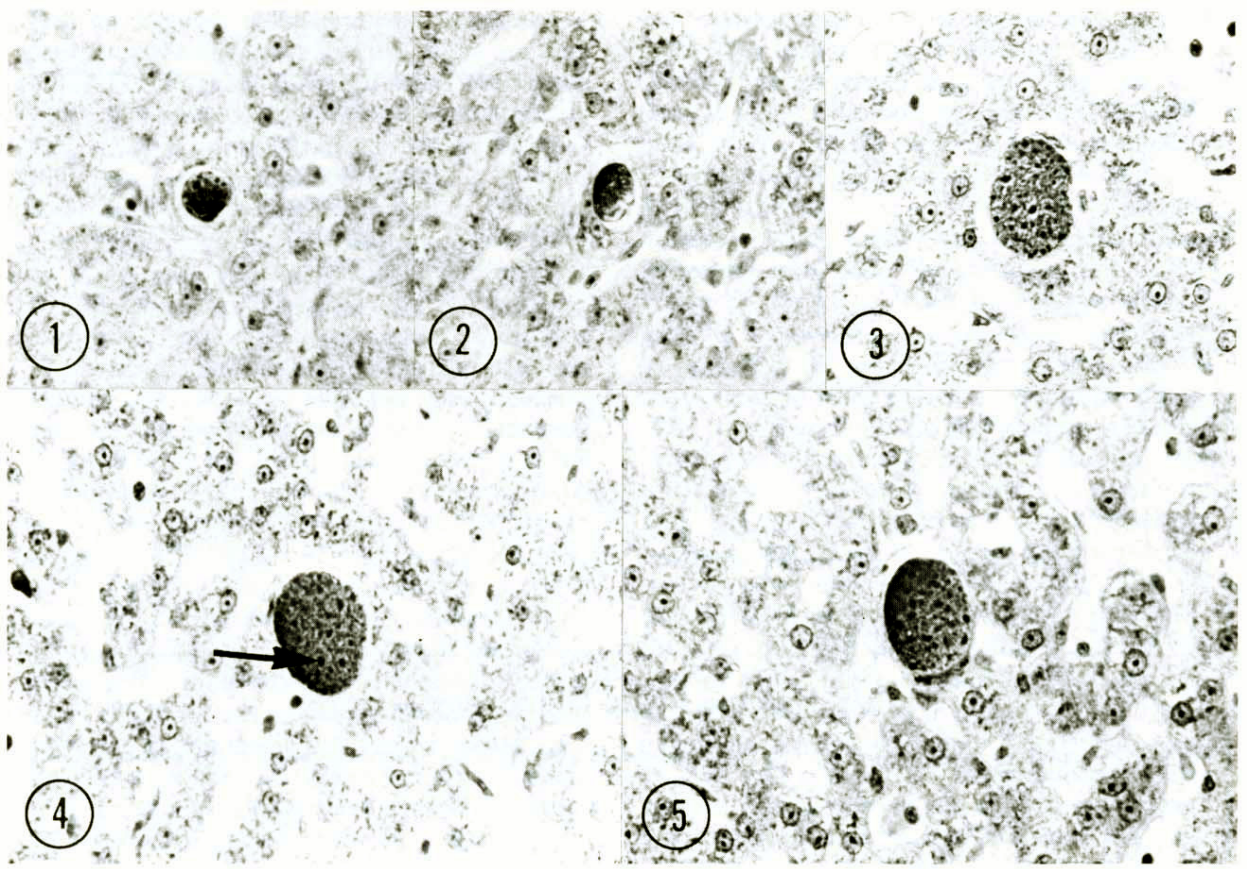

PLATE LIII.—Exoerythrocytic bodies of *Plasmodium knowlesi* in liver tissue of *Macaca mulatta* monkeys. X 580.
Fig. 1. 3-day body.
Fig. 2. 3-day body.
Fig. 3. 4-day body showing numerous flocculi.
Fig. 4. 4-day body.
Fig. 5. 4-day body.

revealed numerous EE bodies at each of the time periods. At 120 hours young ring forms were present in the circulating blood and, at the same time, fully mature EE bodies were demonstrable in the liver sections. The greatest rate of growth appeared to take place between 72 and 96 hours (Plate LIII). Numerous flocculi were present in the sections, but vacuoles were not demonstrable.

It is quite apparent that the EE cycle of *Plasmodium knowlesi,* at least in this strain, is less than 120 hours.

Course of Infection

In the rhesus monkey *(M. mulatta), Plasmodium knowlesi* is a fulminating infection resulting, almost always, in the death of the animal. Studies on sporozoite-induced infections (Fig. 71) show that parasites are first apparent in the peripheral blood by day 6. The median parasitemia curve exhibits a dramatic rise beginning on day 10, which reaches a median infection level of approximately 3.5 parasites per 100 RBC on day 11. At this time, the first animals died. The level of parasitemia continued to rise until day 13, after which it leveled off to approximately 12 parasites per 100 RBC. The mean time of death was 13.6 days with a range of 11 to 16 days.

One of our *M. mulatta* monkeys (T-722) was inoculated with parasitized blood which had been frozen for approximately one year. The infection was slow to develop, not reaching its

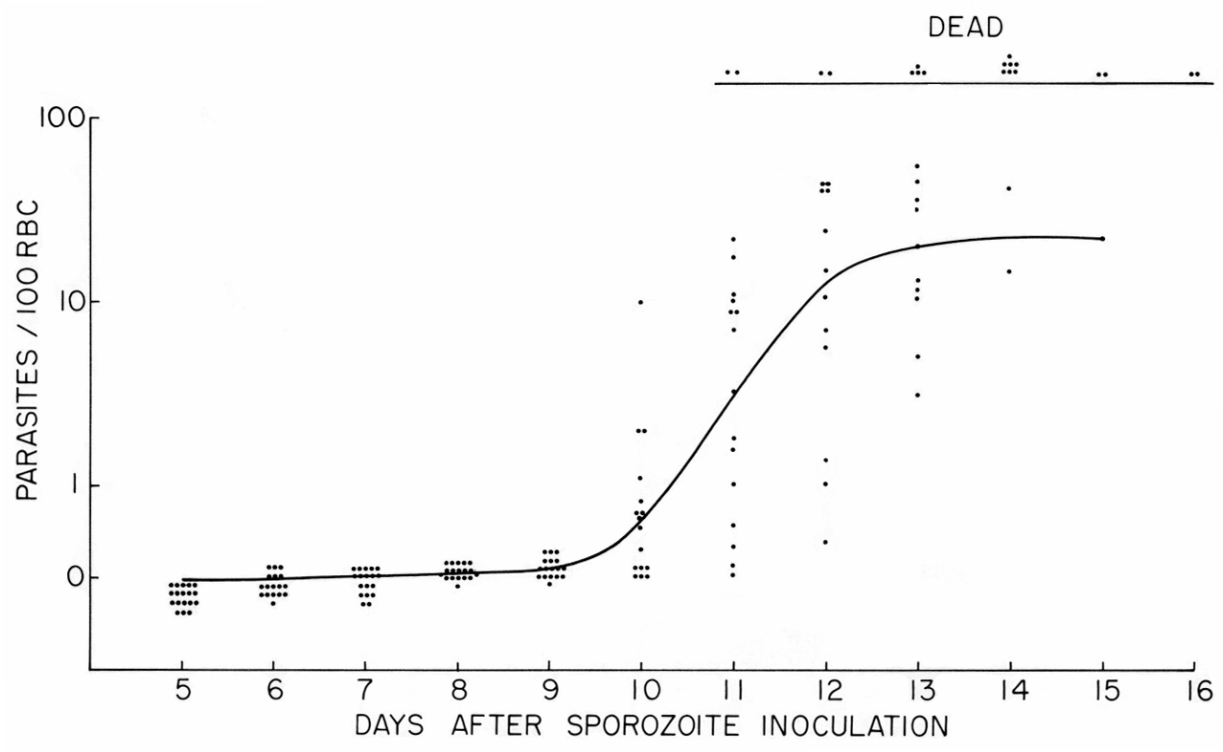

FIGURE 71.—Parasitemia and times of death of 19 *Macaca mulatta* monkeys infected with sporozoites of *Plasmodium knowlesi*.

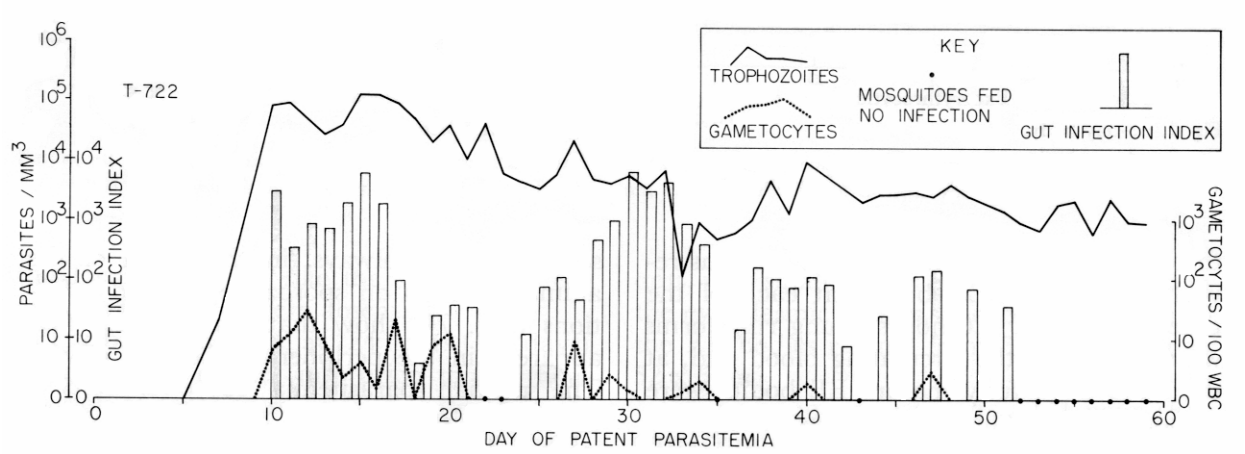

FIGURE 72.—Infectivity of *Plasmodium knowlesi* gametocytes, in a *Macaca mulatta* monkey, to *Anopheles b. balabacensis* mosquitoes.

peak parasitemia until day 15 (Fig. 72) and thereafter slowly declined for the remainder of the 60-day observation period. No treatment was employed. When the nature of the infection became apparent (day 10), daily feeding of *A. b. balabacensis* mosquitoes was initiated and continued, with few interruptions, for the next 50 days. During this period, mosquitoes were infected on 35 of 47 feeding days. There appeared to be 4 distinct waves of mosquito infections which were correlated, partially at least, with gametocytemia.

Plasmodium knowlesi was first shown to infect man by Knowles and Das Gupta (1932) followed by the report of Ionesco-Mihaiesti *et al* (1934). Van Rooyen and Pile (1935) employed

P. knowlesi therapeutically for the treatment of general paresis and reported that non-immunes accepted the infection readily but those with previous experience with *P. vivax* were resistant. An editorial following the von Rooyen-Pile paper called attention to the *loss* of virulence following continued passage in man. The next week, Nicol (1935) in commenting on *P. knowlesi* infection in man mentioned the loss of virulence following man to man passage, also. Chopra and Das Gupta (1936) used *P. knowlesi* transferred directly from a *Silenus rhesus* (= *M. fascicularis)* monkey, for the treatment of neurosyphilis in 2 patients. They were satisfied with the results and pointed out the advantages of the procedure over one employing *P. vivax.* In 1937, Ciuca *et al* published two papers (1937, 1937a) which dealt with a total of 321 patients exposed to infection with *P. knowlesi.* In the first group, probably non-immunes, 79.8 percent developed fever and parasites in their blood. In the second group, most of whom were thought to have had experience with malaria previously, only 46 percent became infected. Following these reports, Ciuca and his colleagues continued to employ *P. knowlesi* for the treatment of general paresis until in 1955 they reported that after 170 transfers, the infection became so virulent it had to be terminated with drugs. Shortly, thereafter, they abandoned the use of the strain. If they were satisfied with the efficacy of the treatment, which is obvious since they continued to use it for so many years, one wonders why they failed to obtain a new isolate, and use it. In contrast to the increased virulence aspect in man encountered by Ciuca *et al,* Jolly *et al* (1937) reported that although *P. knowlesi* produced fulminating infections in their experimental lower animal hosts, it produced only mild infections in *C. papio* after being passed through man. They characterized the disease in man as mild with a tendency toward spontaneous recovery.

Milam and Coggeshall (1938) carried out duration of infection studies in Caucasians and Negroes in this country and produced corroborative evidence as to the mildness and short duration of the initial infection. In general, the infections in Negro patients were milder than those occurring in Caucasians. In the same year, Milam and Kusch (1938) offered *P. knowlesi* infections to a series of 35 patients, of whom 20 had not experienced malaria before, while the remainder had had mild attacks, or had failed to accept infection with *P. vivax.* Included in the series were 6 Negroes. Each of the 29 Caucasian patients developed infections while among the 6 Negroes, 4 experienced only mild infections and 2, none. However, the latter 2 did have low-grade infections because subinoculation of their blood to normal monkeys revealed parasites for up to 3 weeks following their inoculation. Clinically, the course of the disease followed closely that of *P. vivax* except the duration was shorter. Initial fevers were about 102.2° F but later ones had peaks of 104 to 105.8° F which appeared daily for about 10 days and then 'tailed' off to normal. Paroxysms varied from 2 to 15 with an average of 10; definite chills were experienced by only about half of the patients. The highest parasite counts seldom exceeded 100 parasites per 10,000 RBC. However, one patient showed 1,200 parasites per 10,000 RBC. Relapses (recrudescences) occurred which were both clinical and parasitological; they terminated within 3 days.

Through all the work enumerated above, the infection was passed solely by the inoculation of parasitized blood although attempts were made to pass the infection via mosquito bite on occasion (Coggeshall, 1941). Later (1957) Dr. Lainson, according to Garnham (1966) received 90 bites from a lot of *A. labranchiae* mosquitoes, showing 84 percent infected with *P. knowlesi.* Although he was observed for months, no infection developed. The question of transferring *P. knowlesi* to man via mosquito bite, either experimentally or in nature, remained in limbo until a fortunate circumstance occurred in 1965.

Following the accidental sporozoite-induced infection of man in this country with *P. cynomolgi* in May of 1960 (see Chapter 6), investigations were begun in Malaysia where the infecting parasite had originated. That study had several objectives; the one which concerns us here was the possible zoonotic potential of the simian malarias. We were confident this phenomenon could be demonstrated in the field, and the senior author had gone so far as to cast

P. cynomolgi in the starring role. This was not to be, as shown in the following account of an episode which under reasonable circumstances could *not* happen--but did!

In the spring of 1965, a 37 year old American male was detailed by the Army to peninsular Malaysia for a short while and, as part of his assignment, he spent 5 days alone in the bush on Bukit Kertau, working by night and sleeping by day. He returned directly to Kuala Lumpur, the Capitol, and after about a week he left for home. Enroute, he stopped off in Bangkok, Thailand, and on the third morning he felt ill (anorexia, fatigue, and some nausea). He decided home was the best place for him and so he departed. He arrived at the Travis Air Force Base in California on Friday night where he was seen by a base physician. He complained of sore throat, chills, fever, and profuse sweating. He was treated for an upper respiratory infection and departed immediately for his home in Silver Spring, Maryland. He was still sick the next morning (Saturday) whereupon he consulted the family physician. When seen by the doctor, he was having a chill. When questioned, he offered the information that he might have malaria since he had been in Malaysia recently. When his blood smear was examined, the doctor saw only rings and jumped to the conclusion that the patient had falciparum malaria. He told the senior author later, that he did not want to treat the patient because he was unfamiliar with the disease, not having seen a case since his intern days, but remembered that falciparum was deadly.

The doctor decided to refer the patient to the Army's Walter Reed Hospital in Washington, D. C., because the physicians there were familiar with the treatment of the disease and the man was their dependent. Saturday was not an admitting day, and the doctor was told to hold the patient until Monday; this he was afraid to do. He next turned to the NIH Clinical Center in Bethesda, Maryland, where, luckily, the physician on duty was interested in malaria and was well aware of our interest, too. His comment was "send him over." When a blood smear was examined at NIH, some 6 hours later, numerous band forms were in evidence. The diagnosis was *P. malariae.* Because it was known that our group was looking for a strain of *P. malariae,* blood was drawn and (refrigerated) where it remained until sent to our installation at the U.S. Penitentiary in Atlanta, Georgia, on Monday. There it was put into a volunteer who subsequently developed malaria. One can imagine our surprise when the parasite turned out to be *P. knowlesi.* The ring was joined--simian malaria is a zoonosis (see Chin *et al,* 1965). Needless to say, the original patient was cured of his infection and later visited our laboratory on several occasions to fill us in on the many details. One other facet might be mentioned as frosting on the cake. Before the patient left for Malaysia he obtained some chloroquine tablets and later, even though he suspected he had malaria, he refrained from taking them because of an admonition that "drugs should be taken only on advice of a physician." If he had taken one tablet this tale would have died with the parasite.

Subsequent to the original blood-induced infection at the U.S. Penitentiary (Atlanta, Ga.), the disease has been passed, by the same route, 11 times (Chin *et al,* loc. cit. and later) and on 8 occasions by the bites of infected mosquitoes (Chin *et al,* 1968). The daily parasite counts in the volunteers infected by the inoculation of parasitized blood and those infected through the bites of infected mosquitoes showed no appreciable difference, so the data were combined, and are shown in Figure 73 along with the median parasitemia curve. The latter shows that the peak parasite count was reached on day 8 following which the parasitemia fell rapidly to a low level by day 13. Although parasite counts as high as 1200 per mm^3 were encountered as late as the 28th day of parasitemia, most of the patients exhibited no parasitemia after day 16.

The salient features of the blood-induced infections were: the quodidian asexual cycle in the blood, temperatures as high as 104.8° F, and parasite counts as high as 20,850 per mm^3. The clinical manifestations were moderate to severe with attacks terminating spontaneously after two weeks. In the series of sporozoite-induced cases, the course of infection was not much different from that of the blood-induced cases.

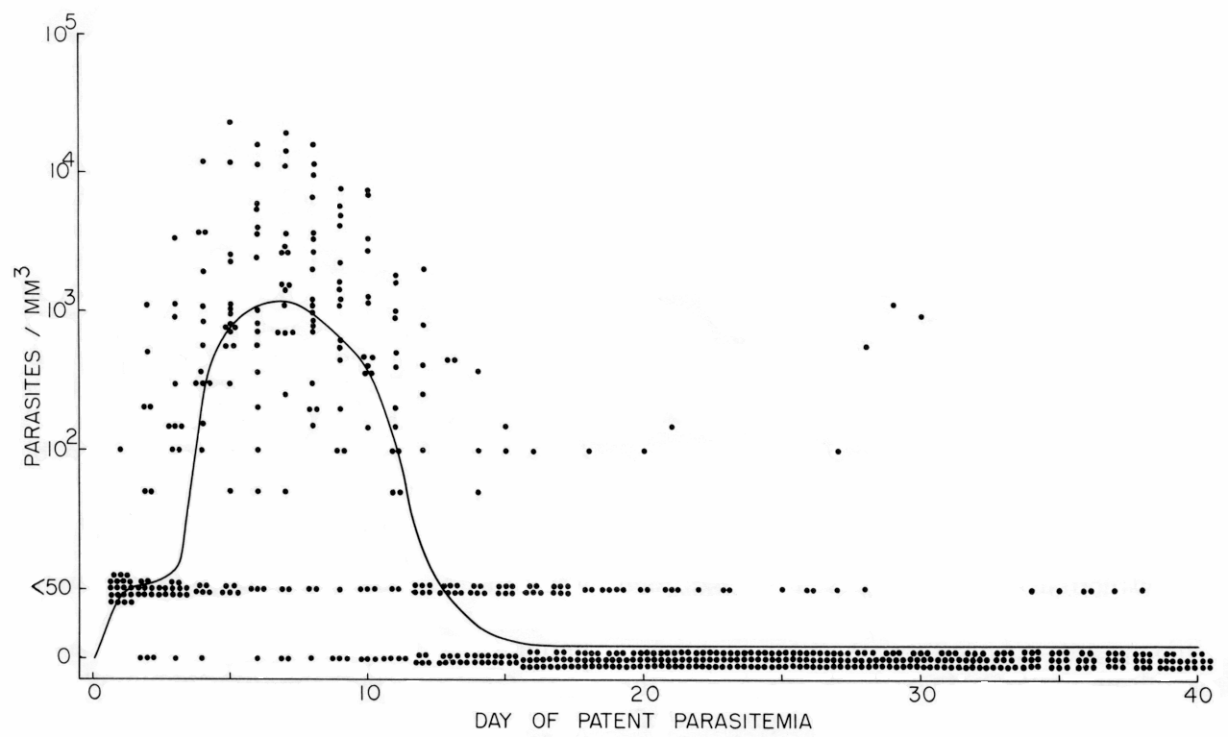

FIGURE 73.—Median parasitemia curve and individual parasite counts in 20 *Plasmodium knowlesi* infections in man (8 sporozoite-induced and 12 blood-induced).

In the main, the data supplied by the investigators who observed the parasite in man agree. At the same time, there are certain points of difference which probably should be mentioned: 1) Van Rooyen and Pile (1935) and Nicol (1935) commented on the loss of virulence when *P. knowlesi* was passaged to man, but the more extensive work of Ciuca *et al* (1955) showed quite the opposite. The work of Chin *et al* lends support to that thesis. 2) Milam and Kusch (1938) remarked about the difficulty of infecting Negroes with *P. knowlesi* but Chin *et al,* in their work, were able to infect Negroes easily and saw no difference between infections in Caucasians and non-whites. What should be stressed is that here, for the first time (Chin and colleagues), *P. knowlesi* was transferred to man by sporozoites, at each attempt, (in one case, following the bite of a single mosquito) with prepatent periods of 9 to 12 days. It is of interest, too, that not only was the infection transferred from man to man via mosquito bites, but also, back, to the rhesus monkey.

Host Specificity

The natural host of *P. knowlesi* is *Macaca irus* (= *fascicularis)* from Malaysia (Sinton & Mulligan, 1932, 1933) and the Philippines (Lambrecht *et al,* 1961). It has also been found in *M. nemestrina* (Eyles *et al,* 1962) and *Presbytis melalophos* (Eyles *et al,* 1962a) in Malaysia.

Experimentally, the parasite readily infects *M. mulatta* as demonstrated by many authors. Experimental infections in other simians are given below:

SPECIES	REFERENCES
Callithrix jacchus	Cruz and de Mello, 1947
Cebus spp.	Garnham, 1966
Cercocebus fuliginosus	Rodhain, 1936
Cercopithecus cephus	Rodhain, 1936
Cercopithecus grisio viridis	Rodhain, 1936
Cynocephalus papio	Jolly, Lavergne and Tanguy, 1937
Hylobates hoolock	Garnham, 1966

SPECIES	REFERENCES
Hylobates lar	Eyles, 1963
Macaca cynomolgus (= *fascicularis*)	Jolly, Lavergne and Tanguy, 1937
Macaca nemstrina	Eyles, 1962
Macaca radiata	unpublished data
Macaca speciosa (= *arctoides*)	Eyles, 1963
Papio doguera	unpublished data
Papio jubilaeus	Rodhain, 1936
Papio papio	Garnham, 1966
Presbytis cristatus	Eyles, 1963
Saimiri sciureus	Chin *et al,* 1965
Semnopithecus entellus	Garnham, 1966

A natural vector of *P. knowlesi* in Malaysia is *Anopheles hackeri* as shown by Wharton and Eyles (1961). In addition, we have found *A. vagus, A. sinensis, A. b. introlatus, A. maculatus, A. kochi, A. b. balabacensis,* and *A. quadrimaculatus* mosquitoes, all but the latter indigenous to peninsular Malaysia, susceptible to infection. Other species which have supported growth of the parasite, at least the presence of oocysts on the gut, are:

SPECIES	REFERENCES
Anopheles annularis	Singh *et al,* 1949
Anopheles atroparvus	Weyer,1937; Hawking *et al,* 1957
Anopheles aztecus	Garnham *et al,* 1957
Anopheles freeborni	Collins *et al,* 1967
Anopheles labranchiae	Garnham, 1966
Anopheles stephensi	Mulligan, 1935; Singh *et al,* 1949; Hawking and Mellanby, 1953; Garnham *et al,* 1957; Hawking *et al,* 1957

Relative susceptibility studies, using eight species of *Anopheles,* (Table 41) indicated that *A. b. balabacensis* was the most susceptible and that *A. albimanus* was refractory to infection. Other species reported to be refractory are *A. fluviatilis* (Singh *et al,* 1950), *A. punctipennis* (Coggeshall, 1941), and *A. subpictus* (Singh *et al,* 1949).

Immunity and Antigenic Relationships

Mulligan and Sinton (1933, 1933a) found that a chronic or latent infection with one strain of *P. knowlesi* conferred an effective immunity against the clinical effects of superinfection with the same strain of parasite. However, such infections did not confer effective immunity against an acute attack following superinfection with a different strain of the same parasite. Multiple heterologous superinfections with certain strains of *P. knowlesi* appeared to produce a marked degree of tolerance to other heterologous strains which had common immunologic factors, but in the absence of such common factors, multiple heterologous superinfections produced no effective tolerance. Shortt *et al* (1938) found that *P. knowlesi* infections which had been cured by administration of drug, gave no residual immunity to infection with the homologous strain of the parasite. Voller and Rossan (1969) were able to show there was no relationship between prior total parasite experience and immunity. A chronic infection, even at a low level, elicited a more effective immunity than frequent cure and challenge. The actual duration of previous parasitemia seemed to be more important than the density of parasitemia in determining the ability of an animal to control infections or to resist challenge.

Brown *et al* (1968) reported that a number of antigenic stabilates are produced during the course of an infection with *P. knowlesi.* It was shown, however, by Voller and Rossan (1969) that although populations of parasites isolated from different recrudescences, of chronic *P. knowlesi* infections, were antigenically distinct, the immunity produced by repeated exposure to one antigenic variant was effective against challenge with heterologous variants.

No cross-immunity between infections due to *P. knowlesi* and those due to *P. cynomolgi* was found by Mulligan and Sinton (1933). Voller *et al* (1966) however, showed that monkeys previously infected with *P. knowlesi* were protected against subsequent challenge with *P. cynomolgi* or *P. coatneyi.* Later work (Voller and Rossan, 1969a) indicated that monkeys with chronic infections of *P. knowlesi,* although refractory to homologous challenge, were susceptible to infection by *P. cynomolgi* and by *P. coatneyi.* Infections of *P. inui* developed somewhat more slowly in monkeys with chronic *P. knowlesi* infections than in control animals.

In man, Ciuca *et al* (1937) demonstrated

79.8 percent of those individuals with little or no previous history of malaria were susceptible to infection with *P. knowlesi.* Of 29 patients subsequently reinoculated with the parasite, none developed a durable infection although a few parasites were found for a limited time. In those individuals with a probable previous history of malaria, the infectivity rate with *P. knowlesi* was only 46 percent. In these patients, a previous infection with *P. knowlesi* gave complete immunity to reinfection. Patients whose first experience was to *P. vivax* displayed only partial resistance to inoculation with *P. knowlesi.*

Antisera to *P. knowlesi* gave a fluorescent antibody cross-reaction at a relatively high level to *P. fieldi* and *P. cynomolgi* antigens (mean reciprocal titer ratios of 100:87 and 100:41), but reacted at a much lower level to other primate malaria antigens (Collins *et al,* 1966). In the reverse procedure, *P. knowlesi* antigen cross-reacted higher to *P. cynomolgi* than it did to *P. fieldi* antisera (mean reciprocal titer ratios of 100:54 versus 100:12).

TABLE 41.—Comparative infectivity of *Plasmodium knowlesi* to eight species of *Anopheles.*

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
Bal						100
Bal : F-1	33	224	946	44.8	23.7	34.7
Bal : Koc	1	26	34	17.1	11.5	19.0
Bal : St-1	19	243	255	35.0	12.9	14.9
Bal : Atro	27	335	379	47.2	15.6	12.3
Bal : Mac	68	1093	2034	38.2	21.7	5.2
Bal : Q-1	24	293	883	52.6	8.0	3.9
Bal : Alb	4	121	88	38.0	0.0	0.0

* Bal = *Anopheles b. balabacensis,* F-1 = *A. freeborni,* Koc = *A. kochi,* St-1 = *A. stephensi,* Atro = *A. atroparvus,* Mac = *A. maculatus,* Q-1 = *A. quadrimaculatus,* Alb = *A. albimanus.*

** GII = Gut Infection Index = average number of oocysts per 100 guts; the GII ratio is the relationship of the GII of the *A. b. balabacensis* to another species where the GII of *A. b. balabacensis* = 100

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(NS) = Not seen